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The 2016 Lasker-DeBakey Clinical Medical Research Award: Innovative hepatitis C virus (HCV) replicons leading to drug development for hepatitis C cure

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The 2016 Lasker-DeBakey Clinical Medical Research Award was given to three scientists working on different stages of the translational sciences on bringing a high efficacious therapy against hepatitis C virus (HCV) infection to a reality. An effective treatment of HCV chronic infection was developed, by a team led by Michael Sofia, using a prodrug approach and the drug PSI-7977 or Sofosbuvir was approved in 2013 less than 28 years after the initial discovery of HCV. This breakthrough was enabled by the availability of the subgenomic HCV replication system, developed by Charles Rice and Ralf Bartenschlager in 1999. The clinically proven Sofosbuvir with remarkable safety and efficacy was designed to enter the hepatocytes readily and to be processed upon cell entry to a triphosphate derivative, becoming a fully activated competitive and high affinity inhibitor for the HCV NS5B, an RNA-dependent RNA polymerase.

While hepatitis diseases as a whole still pose a major public health issue globally, according to a WHO report (WHO reference number: WHO/HIV/2016.06, Global health sector strategy on viral hepatitis, 2016–2021) (http://www.who.int/hepatitis/strategy2016-2021/ghss-hep/en/)) the past few years has witnessed a major breakthrough in the antiviral treatment therapy for hepatitis C. HCV is one of the two viruses, B and C, for causing significant disease burden globally due to the progression to liver cirrhosis or cancers. It culminated recently with Charles Rice and Ralf Bartenschlager, together with Michael Sofia, winning the 2016 Lasker-DeBakey Clinical Medical Research Award. The discoveries of Rice and Bartenschlager led to the development of functional HCV replicons. Sofia harnessed the power of this newly available tool of "live HCV in the lab" for identification and improvement of anti-HCV drugs, leading to the development of a novel molecule using a prodrug approach, now known as Sofosbuvir (PSI-7977).

The milestones in HCV virology and in antiviral drug development are illustrated in Figure 1. The 2016 Lasker Award represents the second time of Lasker Award series recognizing achievement in the HCV field with the 2000 Lasker award on the initial discovery of HCV and the development of screening methods that reduced the risk of its transmission.

BRINGING HCV TO LIFE IN THE LAB: FUNCTIONAL HCV REPLICONS

HCV was the first virus ever discovered by molecular cloning technology in 1989; till then it was grouped in as non-A, non-B hepatitis viruses. The 2016 Lasker Award recognizes the work of Rice and Bartenschlager on the development of HCV replicons in 1999. With the live HCV system estab-

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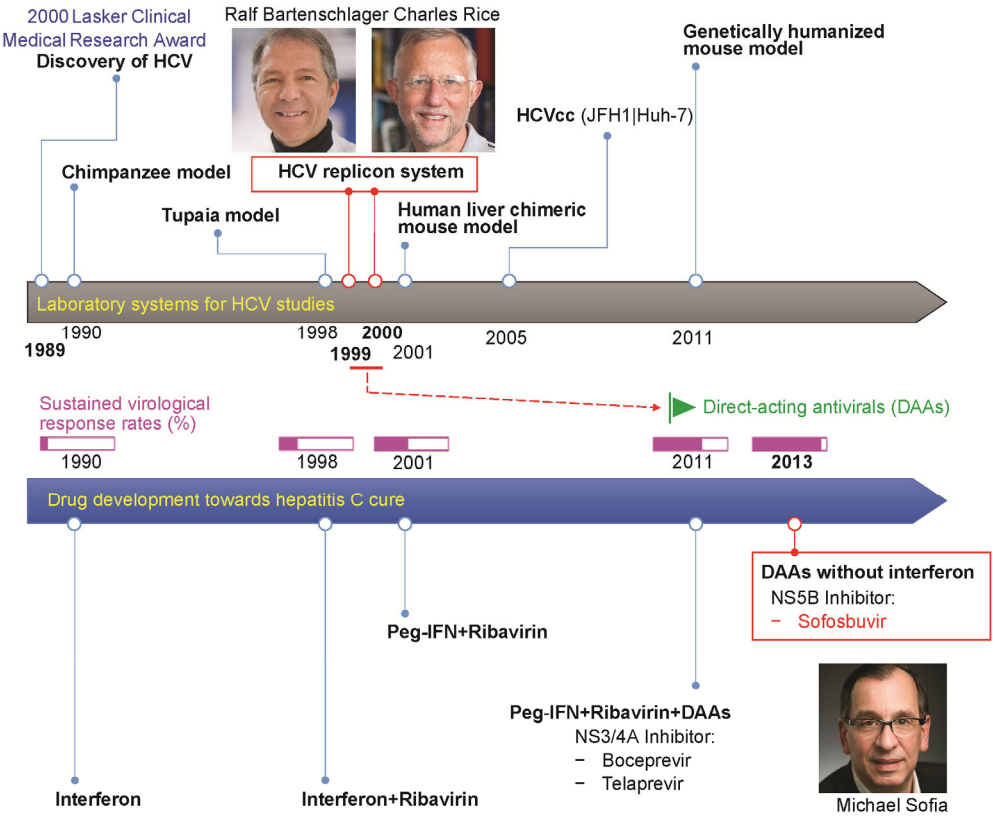

Figure 1 Timeline and milestone in hepatitis C virus (HCV) virology and targeted antiviral drug development. The roles of the 2016 Lasker Awardees, Bartenschlager, Rice, and Sofia, are illustrated. The 2000 Albert Lasker Clinical Medical Research Award was given to Michael Houghton of Chiron and Harvey Alter of NIH for their discovery of HCV (in 1989).

lished in the lab, the viral replication, pathogenesis, and evolution in culture can now be studied with a viable *in vitro* replication system. Functional replicons also enabled the assay development for antiviral drug development.

Before he worked on HCV, Rice was trained in molecular virology of *Flavivirus*. His first HCV paper was published in 1993 on *Journal of Virology* (Grakoui et al., 1993). Since then, he began to shift his research focus to HCV field. His early studies focused on dissecting HCV gene expression and characterizing different viral proteins. In 1996, the Rice Lab determined the conserved 3'-terminal sequence of HCV genomic RNA (Kolykhalov et al., 1996). This finding provided correct terminal sequences that are critically important for recovery of infectious HCV cDNA clone. All previous efforts on making replicons had failed due to the absence of the unanticipated ending sequence. In 1997, the Rice Lab demonstrated that transferring of HCV RNA transcripts derived from full-length cDNA clone into the liver of chimpanzee could successfully cause HCV infection and liver disease (Kolykhalov et al., 1997). This finding enabled the design and generation of a functional virus in lab. In 1999, Bartenschlager reported the successful replication of HCV genomic RNA in a human hepatoma cell line Huh7 (Lohmann et al., 1999). However, the initial system was not efficient for *in vitro* HCV replication. Subsequently, the Rice Lab identified several adaptive mutations in the HCV nonstructural protein NS5A. The new constructs conferred much increased replicative ability *in vitro*, as reported in 2000 (Blight et al., 2000). Continued search on HCV isolates with high *in vitro* replication efficiency yielded the most famous HCV cDNA clone, JFH-1 (genotype 2a). JFH-1 was isolated from a fulminant hepatitis patient by Takaji Wakita (Tokyo Metropolitan Institute of Neuroscience) in 2001 (Kato et al., 2001), exhibiting high efficient replication capability without additional adaptive mutations (Kato et al., 2003). In 2005, studies from Rice and Wakita were simultaneously published to demonstrate the recombinant HCV virus derived from JFH-1 or chimeric genomes based on JFH-1 backbone were infectious in Huh7 cells (Lindenbach et al., 2005; Wakita et al., 2005). Confirmatory reports from various groups worldwide all corroborated the JFH-1 based replicons are robust *in vitro* replication systems supporting complete life cycle of HCV in cell culture.

Another 2016 Lasker Awardee Bartenschlager (and his group) first described the selectable sub-genomic HCV RNA replicon in *Science* (Lohmann et al., 1999), a well-recognized milestone of in HCV cell models. Being a new comer to HCV academic field, Bartenschlager was at the University of Mainz in 1999. Prior to this academic po-

sition, Bartenschlager had finished his PhD at the Center for Molecular Biology in Heidelberg (working on HBV) and his post-doctoral training at Hoffmann-La Roche (working on HCV proteases). Establishing an efficient HCV replicon model had been a goal for years for his group as well as for the Rice group. The replicon was developed by the transfection of the novel abicistronic selectable sub-genomic HCV RNA construct into a human hepatoma cell line Huh-7. They constructed the replicon by introducing a selectable marker, the neomycin phosphotransferase gene, driven by the HCV internal ribosomal entry site, and the HCV non-structural proteins (NS3-NS5B) are expressed from an encephalomyocarditis virus internal ribosomal entry site.

TERMINATING HCV INFECTION IN PATIENTS: DEVELOPMENT OF SOFOSBUVIR

Up to 2012, the best therapeutic regimen was concomitant use of pegylated interferon and ribavirin (or PR regimen). With a 24- or 48-week treatment cycle, the cure rate was 40%–50% with higher cure rate or SVR (Sustained Virologic Response, Figure 1) of 50%–80% for viral genotype 2 and 3 but was less effective for genotype 1. Therefore, efforts continue with the aim to search more effective antiviral drugs with broader genotype coverage and with fewer side effects. One combination, PSI-7797 Sofosbuvir (Sovaldi®, Gilead Sciences, developed by a team led by Sofia) (Sofia et al., 2010) in combination with ribavirin was able to remarkably shorten the treatment cycle to 12 weeks with the cure rate of 80%–95% (Gane et al., 2013).

PSI-7977 was developed based on PSI-6130, a potent antiviral compound. It inhibits the viral RNA replication in a competitive manner by binding to the RNA polymerase NS5B, thus effectively suppressing the viral replication *in vitro* (Stuyver et al., 2006). Due to the poor bioavailability of PSI-6130, the therapeutic effect when tested clinically was not desirable. Sofia and colleagues developed a prodrug version of PSI-6130 which could enter hepatocytes more efficiently. PSI-7977 was designed to enter the hepatocytes readily and then being metabolized into its monophosphate derivative. A further in cell phosphorylation reaction would convert the compound into a triphosphate derivative, which is a potent viral replication inhibitor. The potent antiviral effects were demonstrated in the HCV replicon system (Stuyver et al., 2003), as well as during early phase clinical trials (Figure 1).

This prodrug version, PSI-7977, with remarkable clinical performance in efficacy and safety, was approved in 2013 by USFDA with a commercial name of Sofosbuvir. The performance of Sofosbuvir when used alone was just as remarkable, making the combination therapy with interferon or ribavirin optional. High cure rates were observed for patients with different genotypes with no drug resistance. Up to now, approximately 800,000 patients were treated successfully with Sofosbuvir.

A LONG WAY TO GO FOR REDUCING HCV RELATED MORTALITY

It is certainly great news since so many patients have benefited from this highly accoladed achievements with an approved drug on the market. Given the high efficacy and safety of the newly developed therapy, it would be ideal to treat all patients with chronic HCV infection. It is rather challenging for global accessibility to all the people in needs due to the high pricing. In addition, while the Sofosbuvir based therapy is highly effective in clearing the virus from the body, that may not translate into an immediate effect on lowering the overall disease burden from the public health point of view.

Clearing the virus at a late stage may not be able to halt the progression to cirrhosis and hepato-carcinoma. In addition, there were observations that successful elimination of HCV can reactivate hepatitis B virus (HBV) and could potentially worsen the liver disease in patients with HBV and HCV co-infections. Furthermore, a WHO report showed that the curve in "HBV+HCV" related mortality does not bend until 2020. "Globally, less than 5% of persons living with chronic viral hepatitis are aware of their status", according to the WHO report (WHO reference number: WHO/HIV/2016.06, Global health sector strategy on viral hepatitis, 2016-2021(http://www.who.int/hepatitis/strategy 2016-2021/ghss-hep/en/)). Therefore, it is highly desirable to develop efficacious screening program to call for awareness and treatment. Vaccination, currently available against hepatitis A, B and E, is most effective way to control the virus infection, however, all the efforts on a HCV vaccine development failed so far. In the long run, it is desirable to prevent the HCV infection among general population via vaccination although the mortality of HCV is expected to decrease thanks to the availability of the effective therapy.

CONCLUDING REMARKS

In about 25 years span from the discovery of the virus causing hepatitis C in 1989 to the 2013 approval of Sofosbuvir, an effective drug for its treatment, the progress towards a hepatitis C cure was remarkable. What we can learn from this process is the effective and timely combination and integration of disciplines such as basic science in molecular biology, drug design and development, clinical observations and clinical trials, etc. Millions of lives have been benefited by the effective treatments thanks to the exceptional accomplishments of this year's Lasker Awardees, Bartenschlager, Rice and Sofia. Enabling tools like the functional HCV replicons are the goals for scientists working on other human pathogens such as HBV. The success of the prodrug approach of Sofosbuvir witnesses the power of harnessing the intrinsic metabolic machinery for achieving the targeted therapy with molecules of high specificity, encouraging future rational design of functional biomolecules with targeted delivery and desired metabolic destination.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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